Amendments to the Drawings:

The attached sheets of drawings include a correction of an obvious error in Fig. 2. This sheet replaces the original sheet. In the replacement Fig. 2, the element 5 replaces prior element 25.

The attached sheets of drawings include requested changes to Fig. 3. This sheet replaces the original sheet. In the replacement Fig. 3, the locations of elements 31 and 32 are clarified. In addition, the shaded elements are replaced with unshaded lines.

Attachments: Replacement Sheets 1/7 and 2/7

Annotated Sheets Showing Changes

REMARKS/ARGUMENTS

Amended Drawing:

Figure 2 has been amended to correct an obvious error and a replacement sheet submitted herewith. In Figure 2 of the drawings as filed, the element labeled "25" should have been labeled "5" as substantiated by the following descriptions:

- In [0058] of the specification, referencing Figure 2, it is stated that "The air was introduced at two alternative locations 5 or 6 via a T connection into the static mixer assembly."
- In [0060], it is stated that "In Figure 5A, air was delivered through a T located prior to the first static mixer in the flow path, as depicted at position 5 in figure 2."
- In [0068], it is stated that "In this experiment, air was delivered into the static mixers by means of a stainless steel sparge stone located at first Air Inlet 5."

Corrected Drawing:

Figure 3 has been corrected with a replacement sheet per the Examiner's instruction.

Status of Claims

Claim 16 is amended to correct what is essentially a duplicated term and to further clarify that bubbles are introduced during the lysis step. New claims 33 – 36, dependant from allowed claim 32, are added. Claims 16 - 36 are pending in the application.

Rejections under 35 USC §102

The Examiner's §102 rejection of claims 20 and 21 over Ciccolini, in view of Wan, is respectfully traversed. Claims 20 and 21 have been rejected on the asserted basis that Ciccolini teaches cell lysis in the presence of a controlled stream of gas bubbles. However, Ciccolini does not teach or suggest a process in which cells are lysed in the presence of a controlled stream of gas bubbles as is claimed. Nowhere in Ciccolini is it suggested that either of the two impinging jets for mixing during lysis carry a gas. Indeed, Ciccolini describes these as "reactant streams," which, according to the teaching, would be the cell suspension and the NaOH lysis solution. Ciccolini teaches only addition of air together with potassium acetate at the neutralization step, which is distinct from the lysis step as is claimed. Claim 21 is dependent from claim 20 and, to the extent that claim 20 is not anticipated, claim 21 is likewise not anticipated.

Rejections of claims 16 – 19 under 35 USC §103

The Examiner's §103(a) rejection of claims 16 – 19 over the combination of Cuthbertson (WO99/55837) in view of Theodossiou (*Bioprocess Eng.* 20: 147-156) is respectfully traversed. Claims 16 – 19 are directed to methods for clarifying a bacterial lysate for the ultimate isolation of plasmid DNA, including the steps of introducing a gas into a fluid stream comprising a bacterial cell suspension and a lysis buffer under conditions forming an entrainment of bubbles in the fluid stream during lysis. Claim 16 has been amended to clarify that the gas is added at such time as to be present during lysis. To the extent that any rejection remains in light of this clarification, the following observations are provided.

The Examiner acknowledges that Cuthbertson does not teach any of the claimed lysis, precipitation or separation steps for isolation of plasmid DNA but relies on Theodossiou to teach that a flotation of a bacterial lysates is desirable. Cuthbertson is not a combinable reference because Cuthbertson does not teach or suggest use of a gas (as per the claims) for flotation of lysis floculants. Instead Cuthbertson teaches "pre-prepared encapsulated gas microbubbles," which are both stabilized and derivatizable. Pg. 4, ln 35 Pg 5, lines 14 – 20. More specifically, Cuthbertson teaches use of surface modified gas filled liposomes for targeted isolation, not for nonspecific clarification. Indeed, Cuthbertson distinguishes his separation technique from "flotation separation" using "free gas bubbles or microbubbles generated in situ" which clearly cannot be either stabilized or derivatized and would be unsuitable for the Cuthbertson application, which is essentially affinity purification by flotation. Pg. 4, lns. 30 – 37.

To the extent that Cuthbertson is cited for the proposition that free gas bubbles or microbubbles are used for flotation separation, Cuthbertson states that such methods are used in separation of minerals or the purification of oil-contaminated water, which are clearly non-analogous arts. In order to rely on a reference as a basis for rejection, the reference must be from an analogous art. That is, one that which is either in the field of the applicant's endeavor or is reasonably pertinent to the problem with which the inventor was concerned based on the judgment of a person having ordinary skill in the art. *See* In re Kahn, 441 F.3d 977, 987 (Fed. Cir. 2006).

The addition of either Theodossiou or Levy does not remedy the shortcomings of Cuthbertson. Theodossiou provides little more than the observation that prompted the present inventors to develop

the present claimed invention, to wit, that portions of an alkaline lysate float and these floating flocculants are fragile. Theodossiou teaches that precise control of mixing is required to maximize flocculant flotation but never teaches or suggests the addition of a gas during lysis to effect flotation. The reference to Levy is in fact a review of the Ciccolini reference as discussed above. As previously explained, Ciccolini teaches only use of air during neutralization.

It is noted that Levy specifically teaches away from claim 28 in which mixing is provided by static mixers. Levy states that static mixing per Wan (WO97/23601) will likely cause significant damage to flocculated chromosomal DNA (which would resulting in contamination of plasmid DNA with chromosomal DNA fragments). *See Levy pg. 299, 2nd full paragraph.*

Rejection of claim 22 under 35 USC §103

The Examiner's §103(a) rejection of claim 22 over the combination of Chevalier (WO99/55837) in view of Ciccolini, and Mittlestaedt as evidenced by Theodossiou is respectfully traversed. Claim 22 is directed to a method for purification of extra chromosomal DNA from a bacterial fermentation, including, inter alia, a step of introducing a lysis buffer and a gas into the fluidized stream to form a cell lysate solution comprising a plurality of bubbles. None of the cited references, alone or together, teach or suggest addition of a gas during the lysis step. Indeed, neither Chevalier, Mittlestaedt, or Theodossiou teach or suggest addition of a gas at any time during plasmid purification. As previously discussed, Ciccolini only teaches addition during neutralization. Thus, no combination of these references can be combined to produce the process of claim 22 wherein a gas is added during lysis.

Rejection of claim 23, and claims dependant therefrom, under 35 USC §103

The Examiner's §103(a) rejection of claims 23, 28 and 31 over the combination of Wan (US Patent 5,837,529) in view of Cuthbertson is respectfully traversed. Claim 23 is requires, inter alia, "introducing a suspension of bacterial cells into a fluid flow comprising an alkaline lysis buffer and an entrainment of gas, wherein the cells are flowably mixed with the cell lysis buffer together with the gas thereby forming a cell lysis mixture." As previously discussed, Cuthbertson is not a combinable reference because Cuthbertson does not teach or suggest use of a gas (as per the claims) for flotation of lysis floculants.

It is noted that Levy reiterates the view of one skilled in the art at the time the invention was made that static mixing per Wan will likely cause significant damage to flocculated chromosomal DNA (which would result in contamination of plasmid DNA with chromosomal DNA fragments). *See Levy pg. 299, 2nd full paragraph.* Because it is respectfully urged that 23 is non-obvious, further dependent claims are in turn non-obvious, obviated the need to discuss further citation to the characteristics of spargers.

Applicants surprising found that addition of a gas during lysis through a static mixer resulted in significant purification, not withstanding the conventional wisdom that static mixing would result in perturbation of flocculants with resulting contamination by chromosomal DNA. The applicants' discovery of an efficient method of floating the entire mass of flocculated cellular debris, which permits clarification of the lysate without requiring centrifugation or filtration to remove the floculant is of particular importance in the purification of plasmid DNA at pharmaceutical scale. The methods disclosed and claimed in the present application have been shown to be particularly effective in manufacturing plasmid preparations of high purity and yield at pharmaceutical scale as evidenced by commercial application and success in the field. None of the asserted references teach a method of plasmid or extrachromosomal DNA purification from bacterial cells using a gas introduced during lysis to result in flotation of cell debris thus providing no basis for a motivation to combine with other methodologies for this purpose.

Allowability of claim 32 and claims 29 and 30

The Examiner's determination that claims 32 and 29 and 30 are free of the prior art is gratefully acknowledged. However, because claim 23 from which claims 29 and 30 depend is believed to be patentable, rewriting of claims 29 and 30 has been respectfully deferred.

Conclusion

For the reasons stated herein, the Applicant respectfully submits that independent claims 16, 20, 22 and 23, are allowable and that the dependent claims are, in turn, also allowable. Applicant respectfully requests allowance of the claims at an early date. The Commissioner is authorized to charge any additional fees incurred in this application or credit any overpayment to Deposit

Account No. 50-1922. Should the Examiner have any questions, please do not hesitate to call Applicant's attorney at 832-446-2421.

Respectfully submitted,

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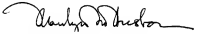
Signature: 
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